ABSTRACT

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Title of diploma thesis: Cloning, expression and purification of selected mycobacterial enzyme

Mycobacterium tuberculosis produces many enzymes which are nescessary for its growing and living. Inhibition of enzymes participating in biosynthesis are a potential target of new drugs in treatement of tuberculosis.

Naphtoate synthase is an mycobacterial enzyme in biosynthetic pathway of menaquinone. It has a crucial meaning in electron transport chain under anaerobic conditions. Naphtoate synthase belongs to crotonase superfamily, catalyses a conversion of *o*-succinylbenzoyl-CoA (OSB-CoA) into 1,4-dihydroxy-2-naphtoyl-CoA (DHNA-CoA).

Plasmid pET-28b(+) and a segment of DNA encoding the sequence of menB amplified by polymerase chain reaction was used for preparation of recombinant protein menB. Ligated plasmid was transformed into the cells *E. coli* HB101 by heat shock. Expression cells *E. colli* BL21 were used for the expression and the expression itself was started by the addition of IPTG. Prepared protein was isolated and purified by a machine Äkta which used the method of affinity chromatography.